

Article

## Introducing Textiles as Material of Construction of Ethanol Bioreactors

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**Abstract:** The conventional materials for constructing bioreactors for ethanol production are stainless and clad carbon steel because of the corrosive behaviour of the fermenting media. As an alternative and cheaper material of construction, a novel textile bioreactor was developed and examined. The textile, coated with several layers to withstand the pressure, resist the chemicals inside the reactor and to be gas-proof was welded to form a 30 L lab reactor. The reactor had excellent performance for fermentative production of bioethanol from sugar using baker's yeast. Experiments with temperature and mixing as process parameters were performed. No bacterial contamination was observed. Bioethanol was produced for all conditions considered with the optimum fermentation time of 15 h and ethanol yield of 0.48 g/g sucrose. The need for mixing and temperature control can be eliminated. Using a textile bioreactor at room temperature of 22 °C without mixing required 2.5 times longer retention time to produce bioethanol than at 30 °C with mixing. This will reduce the fermentation investment cost by 26% for an ethanol plant with capacity of 100,000 m<sup>3</sup> ethanol/y. Also, replacing one 1300 m<sup>3</sup> stainless steel reactor with 1300 m<sup>3</sup> of the textile bioreactor in this plant will reduce the fermentation investment cost by 19%.

**Keywords:** bioethanol; fermentation; bioreactor material; textile bioreactor; reactor cost

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## 1. Introduction

We live in a world where there is an ever-increasing demand for energy. The transportation sector accounts for a high proportion of the global energy demand, which is dominated by fossil fuels [1]. There has been growing interest in alternative fuel sources and ethanol has proven to be a viable alternative to fossil fuel in the transportation sector [2,3]. As the biofuels must compete with fossil fuels, any attempt to reduce their investment and operational costs will contribute to stimulate their consumption.

Global production of ethanol, the dominating biofuel, has increased from 50 million m<sup>3</sup> in 2007 to 89 million m<sup>3</sup> in 2013 [4], the production trends across the globe for this period are shown in Table 1. Future forecast shows that global demand for ethanol will continue to increase to an estimated value of 100 million m<sup>3</sup> in 2015 [5]. For 10% w/w ethanol production in bioreactors, this will correspond to a total fermentation volume of 785 million m<sup>3</sup>. Despite this, the relatively cheaper price of petroleum makes some ways of ethanol production uneconomical, and it is also a hindrance to the commercial introduction of 2<sup>nd</sup> and 3<sup>rd</sup> generation ethanol into the fuel market. Several research projects have been performed on ethanol production to reduce its production costs [6].

**Table 1.** Global ethanol production from 2007 to 2013 by country or region in million m<sup>3</sup> [4].

Country	2007	2008	2009	2010	2011	2012	2013
USA	24.68	35.24	41.4	50.34	52.8	50.35	50.35
Brazil	19	24.5	24.9	26.2	21.1	21.11	23.72
Europe	2.16	2.78	3.94	4.57	4.42	4.46	5.19
China	1.84	1.9	2.05	2.05	2.1	2.1	2.63
Canada	0.8	0.9	1.1	1.35	1.75	1.7	1.98
Rest of World	1.19	1.47	3.46	3.73	2.64	2.85	4.82
World total	49.68	66.79	76.86	88.24	84.81	82.57	88.69

Ethanol is nowadays produced principally by fermentation, where the excess heat of 580 kJ/kg sugar used should be continuously released [6], and the bioreactors must be cooled [7]. In addition, the cost of the fermentation process for a conventional 100,000 m<sup>3</sup>/y ethanol facility constitutes 11% of the total fixed capital cost of the plant [8]. In other word, the fermentation process has a large direct effect on the plant investment and operational costs [8].

A reactor is a vessel where transformation of reactants to products takes place. Reactors are generally designed using the operating conditions for the reactant to product transformation in mind, while also trying to maximise profit, ensure adequate safety and minimize environmental emissions [9]. A fermentor or a bioreactor is a reactor that provides an environment suitable for the controlled growth of a microorganism which is responsible for producing a product of interest [10,11]. A bioreactor should be made of materials that are inert and do not facilitate the development of unwanted microorganisms [12]. It should provide adequate temperature control, operate well under sterilization conditions (with chemicals or temperature) [11], provide good contact area for the microbes and the substrate [9], have adequate charging inlet and discharging outlets, have a means of adequate sampling [11], and provide adequate time for the desired product to be produced [9]. Most conventional

bioreactors are designed to have very low surface area to volume ratio, which increases the cooling requirements of the bioreactor [11].

This paper introduces a novel bioreactor for producing bioethanol made from textiles. The textile bioreactor has the potential for higher flexibility in ethanol production. Its lower cost compared to stainless steel, could lead to a reduction of the cost of producing bioethanol, thereby making investments in the ethanol market more attractive.

## 2. Results and Discussion

The textile bioreactor used in this work is a novel bioreactor for producing bioethanol. It is made from a backbone of textile which is coated with several layers of polymers to make it resistant to chemicals, gas and liquid leakage. It is flexible, long lasting and can withstand temperatures up to 150 °C. Some of the advantages of using a textile bioreactor for bioethanol production include: it does not corrode, it can withstand the tough environmental conditions encountered during fermentation, it is a far cheaper alternative than the currently used bioethanol bioreactors, it is light, and designed for easy and safe transportation, installation and operation, and it is ultraviolet irradiations (UV) resistant, it can be sterilized with steam at 121 °C and with chemicals. It was originally developed for biogas production [13], but it was never examined for any other fermentation products. In this work, this new textile bioreactor was developed for bioethanol production, its performance was examined and the results are presented here.

### 2.1. Textile vs. Other Materials for Construction of Bioreactors

The materials used for constructing bioreactors must be able to withstand the physiochemical conditions encountered while running the bioreactor and during clean-up and sterilization [14]. Apart from stainless steel, other materials that could be used for making bioreactors include carbon steel, borosilicate glass, polytetrafluoroethylene (PTFE) plastic, and ceramics [14]. Only stainless steel 304 and to a lesser extent reinforced carbon steel are currently being used to make industrial ethanol bioreactors. The other materials are normally added to stainless steel bioreactors at specific points for certain purposes (e.g., borosilicate glass used in sight glasses) [14].

Bioethanol is produced by fermentation. Bioethanol fermentation takes place under slightly acidic conditions (pH between 4 and 6), temperatures ranging from 25 to 38 °C, generally without oxygen, and in a liquid medium. Ensuring that only the desired microorganism is what grows in the bioreactor is necessary to ensure the fermentable sugars are converted to the product of interest [15]. It is essential that the material used for constructing bioreactors for producing bioethanol does not affect the fermentation process and can be sterilized when needed. For all the experiments performed in the textile bioreactor, it was autoclaved for sterilization at 121 °C for 20 min and 2 bar pressure. This created a sterile working condition for the textile bioreactor. There were no incidences of bacterial contamination in all the experiments performed in the textile bioreactor, as there were no areas for harbouring unwanted microorganisms, which is one of the main reasons why stainless steel is used as the current material for making bioreactors [16]. The material of construction of the textile bioreactor has been proven to resist diverse environmental conditions (pH 3–12) [13]. In addition the material when burnt does not ignite, but rather forms a semi-solid composite which recoils inward,

making the bioreactor fire resistant. The material was designed to have high tensile strength with high flexibility to make its assembly and disassembly easy. Table 2 shows some advantages and disadvantages of using certain materials of construction for ethanol bioreactors. Considering these features and the comparison in Table 2, as a bioreactor material of construction, the textile bioreactor is an excellent choice for bioethanol production.

**Table 2.** Advantages and disadvantages of possible materials for construction for ethanol bioreactors [14,17,18].

Material	Modification	Advantage	Disadvantage
Textile	Layered with some polymers and UV filter	Portable. Corrosion proof. Good sterility. More cost effective than stainless steel. Can withstand high temperature. Leak proof. Long life span. Can be designed to have regions that are transparent, for easy process monitoring.	Currently a horizontal vessel.
Stainless steel 304	—	Cheapest of all the stainless steel. Leak proof. Good sterility. Can withstand high temperature and pressure. Corrosion proof. Long life span.	Quite expensive.
Carbon steel	Reinforced with stainless steel	Cheaper than 304 stainless steel. Leak proof.	Corrosion and contamination.
Borosilicate glass	—	Transparent. Inert to chemicals.	Very fragile.
Plastic	—	Very portable. Cheap.	Leaks and short life span. High chances of contamination.
Ceramics	—	Chemically stable. Wear resistant.	Brittle. Prone to thermal shock.

## 2.2. Reactor Cost Comparison

A major challenge facing biofuel production is its economic feasibility [19]. Bioethanol production consists of the collection of feedstock, pre-treatment of feedstock (if the feedstock is starch or lignocellulosic based), fermentation, distillation and possibly dehydration [20]. The fermentation cost of a 100,000 m<sup>3</sup>/y ethanol production facility contributes 11% of the total plant cost, while the bioreactor cost makes up 32% of the fermentation cost [8]. In this section a comparison is made between the investment cost of stainless steel bioreactors and textile bioreactors excluding operation cost (maintenance and installation cost). Typically, the installed cost (investment and operation cost) of a stainless steel reactor is 1.7 times its purchase cost [21], while that of a textile bioreactor is 1.5 times its purchase cost [13].

The purchase cost for a 1000 m<sup>3</sup> textile bioreactor is \$100,000. Table 3 shows the purchasing cost of different reactor sizes, for both the developed textile bioreactor and stainless steel reactors. The purchasing cost of stainless steel reactor was estimated using Equations (1) and (2) (see Section 3.5). For all reactor volumes considered, the purchasing cost of the developed textile bioreactor was far less than half the purchasing cost of the stainless steel bioreactor. Considering a 100,000 m<sup>3</sup>/y ethanol production facility using sucrose as its raw material and having a fermentation time between 10 and 15 h [22,23], this plant will require a bioreactor volume between 1000 and 1500 m<sup>3</sup> for the fermentation only. If this plant has just one 1300 m<sup>3</sup> stainless steel bioreactor, replacing this with a 1300 m<sup>3</sup> textile bioreactor will reduce the fermentation investment cost by 19%, and the total plant investment cost of the facility by 2.1%.

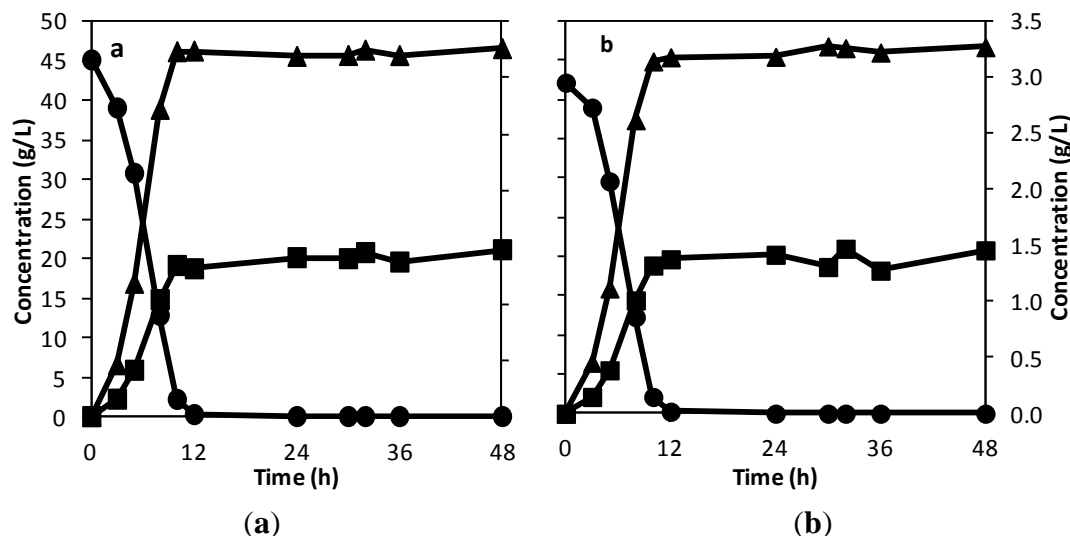
**Table 3.** Purchasing cost of developed textile bioreactors and 304 stainless steel reactors.

Reactor Size (m <sup>3</sup> )	Purchasing Cost of Developed Textile Bioreactor (\$)	Purchasing Cost of 304 Stainless Steel Reactor (\$)
500	66,000	201,000
1,000	100,000	282,000
1,300	130,000	325,000
1,500	150,000	352,000

### 2.3. Mixing and Temperature Control in the Textile Bioreactor

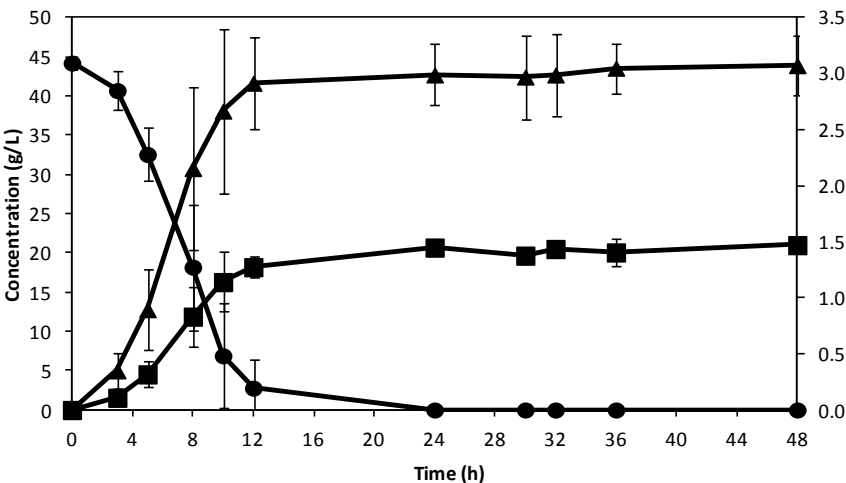
The mass transfer in a bioreactor affects the net productivity of the system [11,24]. The two crucial aspects of mass transfer in a bioreactor are the uniform distribution of the product and substrate in the bulk liquid, and the transfer of substrate into the cells and the products out of the cells. Mixing helps to minimize local variation of concentration and temperature in a bioreactor [11]. Mixing in liquid media can be achieved by agitation, or with the aid of a stirrer, or by the use of a pump for recirculation, depending on the viscosity of the liquid and if it media is single- or multi-phased [25]. For the textile bioreactor, mixing was performed using a recirculation pump. To determine the effectiveness of the mixing in the bioreactor and the possibility of it being used for continuous fermentation, experiments were performed where samples were collected from the sampling point at the centre of the textile bioreactor and from the exit pipes from the bioreactor. One of the basics of a continuous stirred tank reactor (CSTR) is having same concentration in the reactor as what leaves the reactor. From Figure 1, it is clearly observed that there is no significance difference between the concentration in the bioreactor (samples from the centre of the reactor) and that leaving the bioreactor (samples from the exit pipe). This shows that there is the possibility of the textile bioreactor being used for batch, fed-batch, and continuous fermentation. In addition to ensuring uniform substrate and product distribution in the textile bioreactor, the mixing also helped to provide a good transfer of substrate into and products out of the yeast (Figure 1), as the sugar was fully consumed about the same time as peak ethanol concentration was reached. Thus the mixing by recirculation in the textile bioreactor is effective.

**Figure 1.** Concentration of sucrose (●) and ethanol (■) on the primary axis (left side), and glycerol (▲) on the secondary axis (right side), with samples taken from the exit pipe (a) and centre of the reactor (b) with time, to determine the effectiveness of mixing by recirculation.

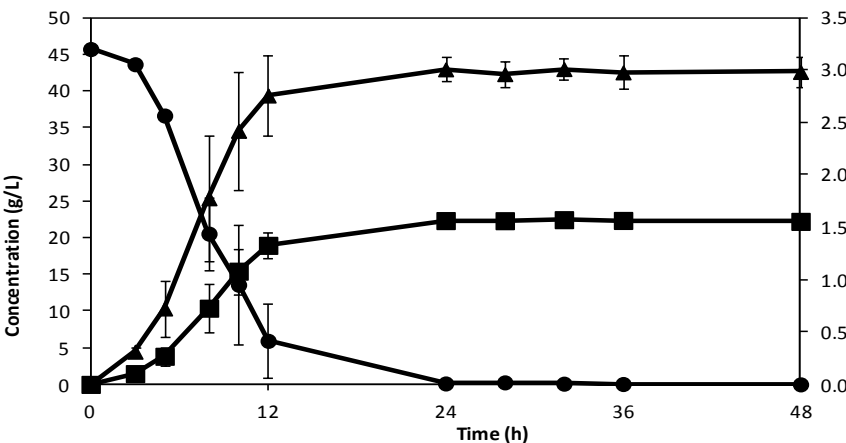


Temperature control is essential for optimal product formation as every microorganism has a temperature range in which it functions optimally. For anaerobic condition that for *S. cerevisiae* is around 30 °C [15]. For lab scale production heat is normally added to the system while for the large industrial bioreactors with low surface to volume ratio, cooling is necessary [11]. Because of the nature of the material used for the developed textile bioreactor, cooling can easily be achieved by recirculation of chilled water, while heating can be achieved with hot water. The area to volume ratio of a 1000 m<sup>3</sup> textile bioreactor is 0.96, while that of a conventional 1000 m<sup>3</sup> bioreactor having a height to diameter ratio of 3 it is 0.62. The higher area to volume ratio of the textile bioreactor makes cooling (or heating) easily achievable because the heat loss (or gained) by evaporation and radiation increases with increasing area to volume ratio. Temperature control was achieved in the textile bioreactor as described in Section 3.2. In addition, the recirculation of the fluid also helped to ensure temperature uniformity in every part of the reactor. Figures 2 and 3 shows the result of the experiments performed with temperature control, while Figure 4 shows the result of the experiments without temperature control. For both cases, ethanol yields greater than 87% of the theoretical values were reached, while the experiments where temperature was maintained at 30 °C had higher fermentation rates and peak product concentrations were reached in less than 24 h. This shows that the temperature control developed for the textile bioreactor is effective.

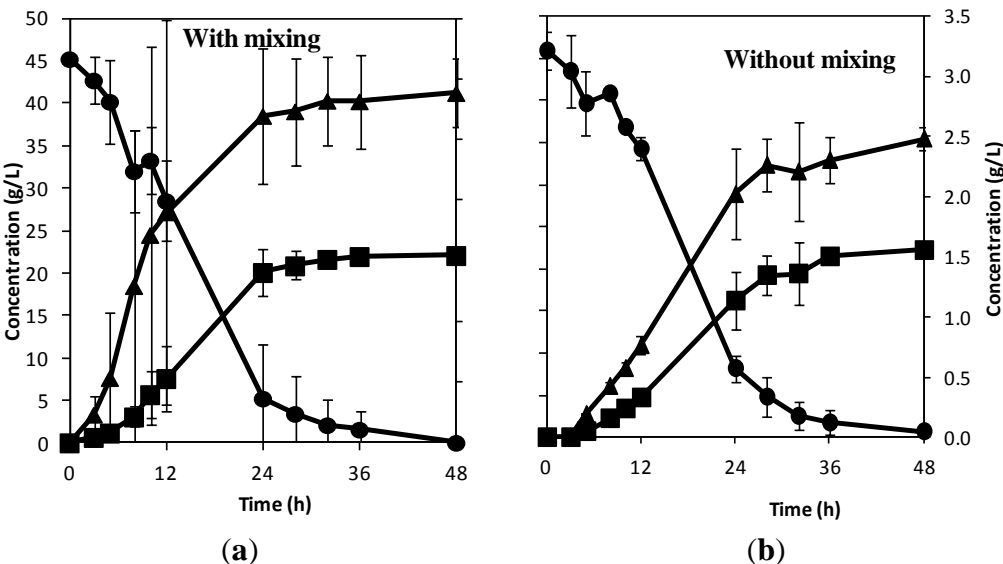
**Figure 2.** Concentration of sucrose (●) and ethanol (■) on the primary axis, and glycerol (▲) on the secondary axis with time, at 30 °C and with mixing.



**Figure 3.** Concentration of sucrose (●) and ethanol (■) on the primary axis, and glycerol (▲) on the secondary axis with time, at 30 °C without mixing.



**Figure 4.** Concentration of sucrose (●) and ethanol (■) on the primary axis, and glycerol (▲) on the secondary axis with time, at room temperature of 22 °C (a) with and (b) without mixing.



#### 2.4. Fermentation in the Textile Bioreactor and Its Economics

To determine how effective the textile bioreactor was for producing bioethanol, lab scale experiments were performed under different operating conditions. Temperature and mixing were varied, while the pH of all the experiments performed was around  $6.0 \pm 0.2$ . Figure 2 shows the result of the experiment performed in the textile bioreactor at 30 °C and with mixing. For this experiment, the yield of ethanol from the experiment (using the initial sucrose concentration of 44.2 g/L) was  $0.48 \pm 0.01$  g/g, which is 88% of the theoretical value, and it took an average of 15 h for the yeast to consume the sugar. Comparing this fermentation time with that from a similar work where 10 g/L of yeast was used and it required a fermentation time of 10 h [22], shows that the fermentation time is good. Thus fermentation takes place effectively well and at a good rate in the textile bioreactor. From Figure 2, the average of the peak ethanol concentration was  $20.04 \pm 0.53$  g/L, using the average fermentation time gave the specific productivity to be  $1.34 \pm 0.02$  g L<sup>-1</sup> h<sup>-1</sup>.

Figure 3 shows the result of the experiment where temperature was fixed at 30 °C without mixing. The yield of ethanol from the experiment was  $0.49 \pm 0.01$  g/g, and it took an average of 20 h for the yeast to consume the sugar. Comparing Figures 2 and 3 shows that mixing did not affect the fermentation rate that much when temperature is held at 30 °C without mixing, as the specific productivity for this case was  $1.04 \pm 0.01$  g L<sup>-1</sup> h<sup>-1</sup> in comparison to  $1.34 \pm 0.02$  g L<sup>-1</sup> h<sup>-1</sup> with mixing. To produce the same amount of ethanol as that which is produced when mixing is controlled; the textile bioreactor volume used has to be 1.29 times the one used with mixing. Taking a 1000 m<sup>3</sup> bioreactor operating with mixing and temperature control, this bioreactor will cost \$282,000 but a 1300 m<sup>3</sup> textile bioreactor will cost \$130,000 (see Table 3). In addition, the cost of agitation and mixing in a bioreactor accounts for 24% of the fermentation cost of a 100,000 m<sup>3</sup> ethanol/y production facility [8]. Using a textile bioreactor operated at 30 °C without mixing can eliminate the need for the cost of agitation and mixing, and it gives a bioreactor cost reduction of \$152,000.

Figure 4 shows the result of the experiment that was performed at room temperature of 22 °C with and without mixing. The ethanol yield from the experiment with mixing was  $0.49 \pm 0.01$  g/g, and it took an average of 40 h for the yeast to consume the sugar, while that without mixing had an ethanol yield of  $0.49 \pm 0.02$  g/g, and it took an average of 42 h for peak ethanol concentration to be reached. Mixing did not affect the fermentation rate that much as the specific productivity with mixing was  $0.55 \pm 0.01$  g L<sup>-1</sup> h<sup>-1</sup> while that without mixing was  $0.53 \pm 0.02$  g L<sup>-1</sup> h<sup>-1</sup>. This result shows that there is a possibility of running the textile bioreactor without temperature control and mixing. The slower fermentation rate from producing bioethanol at 22 °C can be accommodated by increasing the retention time and the bioreactor volume. Comparing Figures 2 and 4, the same amount of ethanol per hour will be produced in both cases if the volume of the textile bioreactor for the production without temperature control and mixing is 2.53 times that with temperature control and mixing. Taking a 1000 m<sup>3</sup> bioreactor with temperature control and mixing, the purchasing cost of a 1000 m<sup>3</sup> bioreactor is \$282,000 while that of a 2530 m<sup>3</sup> textile bioreactor (consisting of one 1000 m<sup>3</sup> reactor and one 1500 m<sup>3</sup> reactor) is \$250,000 (see Table 3). In addition, operating the textile bioreactor without temperature control and mixing also reduces the total fermentation cost, as the cost of temperature control, mixing and agitation accounts for 26% of the fermentation cost in a 100,000 m<sup>3</sup>/y ethanol production plant [8]. For the same bioethanol production rate it is more economical to use a larger



volume of the textile reactor without temperature control and mixing than a smaller bioreactor volume with temperature control, mixing and agitation.

Comparing the scenario where there is mixing but the textile bioreactor is operated at 22 °C, the size of the reactor in this case would be 2.44 times the size of that operated at 30 °C. Using a 1000 m<sup>3</sup> bioreactor at 30 °C will cost \$282,000 while a 2440 m<sup>3</sup> textile bioreactor running at 22 °C will cost \$250,000. However running the textile bioreactor at 22 °C with mixing will only reduce the fermentation cost by 2%, which is not as economical as 26% cost reduction obtained by running it at 22 °C without mixing [8].

For all experiments performed in the textile bioreactor there were no incidences of bacterial contamination. From the results, experiments performed at 30 °C had faster fermentation rates than the ones performed at room temperature. For a continuous process, both temperature control and mixing will be essential to achieve high dilution rate. The results of the experiments show that the textile bioreactor can be used for bioethanol production at different conditions of temperature and mixing.

### 3. Experimental Section

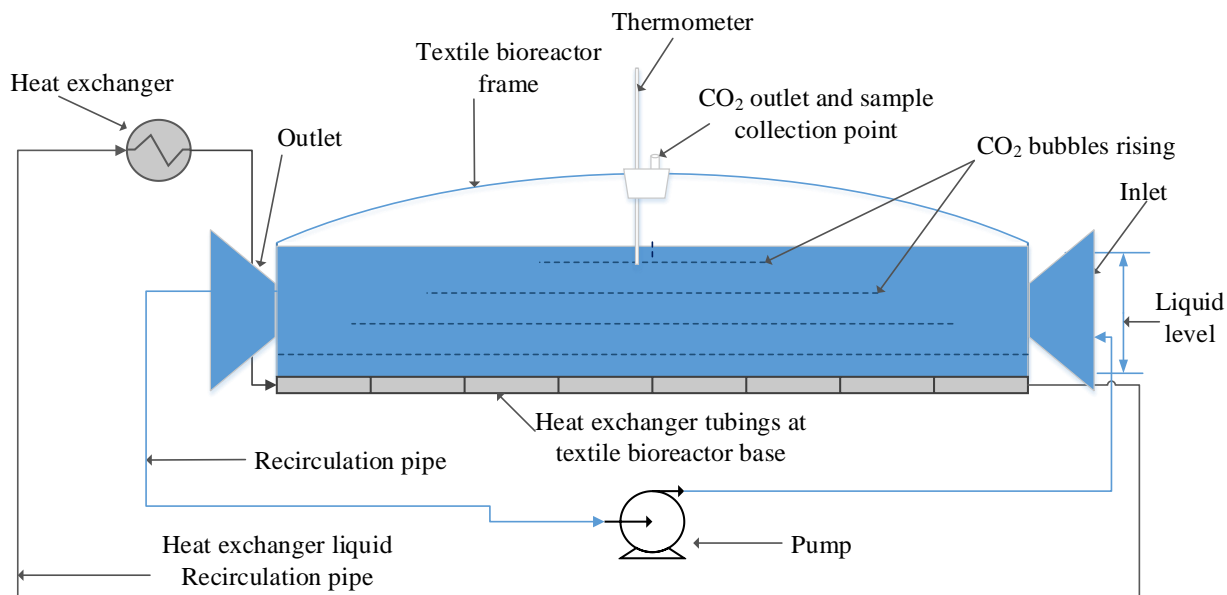
#### 3.1. Microorganism

Dry ethanol red yeast (*Saccharomyces cerevisiae*) from Fermentis (Strasbourg, France) was used for the fermentation. A starting concentration of 1 g/L of the dry yeast was used.

#### 3.2. Textile Bioreactor

A schematic of the lab scale prototype of the developed bioreactor is shown in Figure 5. The material of construction (MOC) was a textile backbone coated with several layers to protect against pressure, chemicals in the reactor, weather conditions and to be gas proof. The material was developed by FOV Fabrics AB (Borås, Sweden) primarily for biogas reactors and it was welded to form a reactor by Kungsäter Industri AB (Kungsäter, Sweden). The lab scale bioreactor had a total volume of 30 L and a working volume of 25 L. The dimensions of the bioreactor were 100 cm length, 50 cm breadth, and 6 cm width. It had an opening of 4 cm diameter, which serves for sample collection; probe stand, thermometer stand, and gas exit. The dimensions of the outlet and inlet tapered from 9 to 4 cm at the bioreactor entrance, to allow for easy loading of the bioreactor. The dimension of the tubes connected to the inlet and outlet were 8 mm.

The means for temperature control was made using Poly(vinyl chloride) (PVC) tubing of 50 m length and a woollen blanket; the PVC tubing was connected to a GD120 grant thermostatic circulator (GD Grant Instruments Ltd., Cambridge, UK). The tubing was wound 14 times, covering the whole perimeter of the textile bioreactor at the bottom, as such, *Saccharomyces cerevisiae* was not exposed to thermal shocks [12]. The tubing and the textile bioreactor were enclosed by the woollen blanket. The temperature of the thermostatic circulator was set at 33 °C. A 200 rpm Watson Marlow compact peristaltic pump (W-M Alitea AB, Stockholm, Sweden) was used for recirculation of the fluid in the reactor for mixing.

**Figure 5.** Schematic diagram of the textile bioreactor lab scale prototype setup.

### 3.3. Experimental setup

Experiments with temperature (30 °C and room temperature of 22 °C) and (with and without) mixing using recirculation as process variables were carried out in the textile bioreactor. Sucrose (50 g/L) was used as the carbon and energy sources, supplemented with 7.5 g/L  $(\text{NH}_4)_2\text{SO}_4$ , 3.5 g/L  $\text{KH}_2\text{PO}_4$ , 0.75 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 1.0 g/L yeast extract. The total liquid volume in the reactor was 25 L for all experiments. The flow rate used for recirculation of the fluid for the experiments with mixing was 0.924 L/min. Sucrose concentration dropped between 44 and 47 g/L when the feed stream used for each experiment was autoclaved. Each experiment was performed in duplicate.

To determine the effectiveness of the mixing in the bioreactor, samples were taken from the recirculation pipe and from the centre of the reactor.

### 3.4. Analytical Method

Liquid samples from the textile bioreactor were analysed using high-performance liquid chromatography (HPLC) with a hydrogen-based ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) at 60 °C and 5 mM 0.6 mL/min  $\text{H}_2\text{SO}_4$  eluent. A refractive index detector (Waters 2414, Waters Corporation, Milford, MA, USA) and a UV detector (Waters 2487) were used with the HPLC. The samples used for the HPLC analysis were centrifuged for 10 min at 10,000  $\times g$  and the liquid portion stored for analysis. The samples were stored at −20 °C prior to HPLC analysis. All reported error bars and interval represents two standard deviations. The yield for each experiment was calculated using the concentrations measured by the HPLC after autoclaving.

### 3.5. Cost Estimation

The purchasing cost of 500, 1000, 1300 and 1500 m<sup>3</sup> versions of the developed textile bioreactors were provided by FOV Fabrics AB, and compared with those of 304 stainless steel reactors.

The purchasing cost of a 304 steel reactor was estimated using Equation (1) [21], where  $V$  is the reactor volume in gallons and  $F_m$  is 2.4 for 304 stainless steel [21]. The Chemical Engineering Plant Cost Index (CEPCI) as at when Equation (1) was developed is 325.8 [21]. The capital cost was then updated to January 2014 values using Chemical Engineering Plant Cost Index (CEPCI) of 572.8 [26]. The updated cost was computed using Equation (2):

$$C = F_m \exp[11.662 - 0.6104(\ln V) + 0.04536(\ln V)^2] \quad (1)$$

$$C_{\text{updated}} = C (I_{\text{updated}}/I) \quad (2)$$

#### 4. Conclusions

In this work, a novel bioreactor for bioethanol production was introduced. The bioreactor has textile as its core material of construction. For the experiments performed on a lab scale prototype textile bioreactor, the optimum result for possible continuous production of bioethanol was that obtained from the experiment performed at 30 °C and with mixing, having a yield of  $0.48 \pm 0.01$  g/g and it took an average of 15 h for all the sugar to be fermented and peak bioethanol production level to be reached. For the same ethanol production rate, the need for mixing and temperature control can be eliminated by using a textile bioreactor 2.5 times the volume of that needed with temperature and mixing control. Doing this For a 100,000 m<sup>3</sup>/y bioethanol production facility will reduce the fermentation investment cost by 26%, while replacing a 1300 m<sup>3</sup> stainless steel reactor with a 1300 m<sup>3</sup> textile bioreactor running at 30 °C and with mixing will reduce the fermentation investment cost by 19% and the total plant investment cost by 2.1%.

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#### Author Contributions

Osagie A. Osadolor did the experimental design and performed all the experiments, wrote most of the manuscript and was responsible for part of the idea. Patrik R. Lennartsson was responsible for part of the idea and manuscript. Mohammad J. Taherzadeh was responsible for part of the idea and manuscript. All authors have given their approval to the final version of the manuscript.

#### Conflicts of Interest

The authors declare no conflict of interest.

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